

The effect of Temperature on The
Rate of Movement in the Marine
Ameba, Mayorella Conipes.

-by-

Fred W. Allen Jr.

University of Kansas A.B. '26.

Submitted to the Department of
Zoology and the Faculty of the
University of Kansas in Partial
fulfillment of the requirements
for the degree of:-

Master of Arts

Approved by:-

Asa A. Schaeffer
Instructor in Charge

H. H. Lane
Head or Chairman of Dept.

May 27, 1927.
(Date)

1. ACKNOWLEDGEMENTS
2. INTRODUCTION
3. HISTORICAL
4. MATERIAL
5. METHODS
6. RESULTS
7. CONCLUSIONS
8. LITERATURE CITED

ACKNOWLEDGEMENTS

I wish to express my appreciation to Dr. A. A. Schaeffer for his helpful suggestions and for the personal interest he has shown throughout this most interesting problem.

INTRODUCTION

It is the purpose of this paper to show the effect of temperature changes on the activity of *Mayorella conipes*, a small marine ameba. By a consideration of (1) the velocity at constant temperatures, (2) the velocity at various temperatures observed on the same ameba, (3) the fall in rate above the optimum, (4) the effect of successive rises and falls in the temperature, (5) rhythmic activity, (6) general effect of temperature on movement, an attempt will be made to construct its temperature curve. These data will then be used to construct its temperature curve, and as a basis of comparison with other forms.

HISTORY

Ameboid movement was first discovered by Rosel v. Rosenhof in 1755. While this peculiar type of movement was interesting it was not considered important because of its seemingly isolated and limited occurrence. Although Dujardin in 1841 from his studies on the pseudopods of amebas and foraminiferas made the remarkable discovery that protoplasm is the physical basis of life, he contributed little or nothing to the question of ameboid movement.

It was not until 1774 that Corti noticed a similar streaming of protoplasm in the cells of *Chara*. The difference being that no locomotion accompanied the streaming in the cells of *Chara*, but was always found in ameba. This seemed to be the only stimulus necessary for the large amount of investigation that followed. From these facts came the generally accepted view that "the really fundamental feature of ameboid movement is the streaming of the protoplasm" (Schaeffer '20).

The streaming of protoplasm was found to be the important element

in connection with ameboid movement, and was the source of a large amount of investigation. The physiologists soon noticed the ameboid character of the white blood cells, and later followed the discovery of ameboid movement among other animals and animal cells. So widespread has the observation of ameboid movement become that out of it has grown the theory "that even muscular movement is at bottom a specialized sort of ameboid movement, not merely phylogenetically but as it is now known" (Schaeffer '20).

Many theories have been proposed to explain ameboid movement. Most of these theories concern themselves, however, with the accessory phenomena, rather than the central processes that lie at the bottom of ameboid movement (streaming).

Streaming always occurs in ameboid movement but some of the accessory phenomena such as surface tension layer and the formation of the ectoplasm that are said to lie at the bottom of ameboid movement do not always occur. We need rather than theories, a large amount of objective data to solve the question of ameboid movement.

In discussing the third layer or the surface tension layer in amebas, Schaeffer (26) questions its importance as a necessary factor in ameboid movement since it does not move the same way in the pseudopods of *Diffugia* or of *Foraminifera* and is entirely lacking as a moving layer in ciliates and plant cells where streaming protoplasm occurs. He never-the-less recognized that its movements indicate a greater surface tension at the ends of active pseudopods than over the rest of the ameba and suggests that "this fact if correctly interpreted, may prove to be a useful guide in further experimental analysis".

When amebas move, free from external stimuli, in a clear fluid, they move in a wavy or sinusoidal path, which represents a helical spiral path

on a plane surface. This spiral movement is not confined to amebas but occurs in all animals including man and all motile plants. Larvae of many species of aquatic invertebrates and all motile spermatozoa move in a spiral path. The spiral path of the ameba is important since it has been found that this path is a projection of the well known helical spiral path of ciliates and flagellates on a plane surface, since the ameba is restricted in its movement to two dimensions of space (Schaeffer '20).

Bullington ('25) in his study of 165 ciliates and one suctorian concluded that "all ciliates swim in a spiral path and that the spiral is characteristic for each species". The investigation showed no correlation between any visible morphological structure and the direction of turning.

Because so many plants and animals of such widely separated groups move in spirals, Schaeffer ('20) thinks that spiral movement is not an acquired habit to overcome asymmetry of the body but believes the cause for its existence to be found in phylogeny. He was the first to break away from the older idea that the reactions of animals were due to tropisms or chemical or physical stimuli exclusively. He thinks instead that there is an "automatic regulating mechanism" present in every living organism, comparable to the brain centers of higher animals.

Rogers ('27) suggests that something may be learned as to the nature of a physiological process from a study of the effect of temperature on that process. He bases this, of course, on the well known law of Van't Hoff which states that the velocity of a chemical reaction is approximately doubled or trebled by a rise in temperature of 10° C. This law has been found to be valid for various kinds of biological processes

by a number of investigators. Kantz studies the frequency of contraction in the isolated heart of rabbit and dog. Snyder investigated such phenomena as the rate of transmission of nerve impulses in the pike and frog, and the rhythmic contraction of mammalian small intestine. In all of these cases the velocity was found to be increased two or three times by a rise in temperature of 10°C .

Strumpter reports that in the case of ants, these insects move twice as fast at 21°C as they do at 11°C . McCutcheon from his studies on the locomotion of leucocytes of *Limulus* found the velocity of the locomotion shown by neutrophillic polymorphonuclear leucocytes of one individual to be doubled by a rise in temperature of 10°C within certain limits of temperature variation.

In the above cases the Van't Hoff law seems to be followed, and we are justified in thinking with Rogers that "if any physiological process shows essentially the same modification in rate by changes of temperature as do ordinary chemical reactions the belief would seem to be justifiable that some chemical reaction lies at the basis of this physiological process".

Pantin, ('24) in his work on the "Physiology of Amoeboid Movement" showed that the rate of change of state ($\text{sol} \rightleftharpoons \text{gel}$) in the protoplasm of amebas is directly related to the velocity. From this it is argued that the velocity of continuous amoeboid movement does not depend directly upon the velocity of some chemical reaction supplying the necessary energy, but it probably does depend on the rate at which the protoplasm can change its state ($\text{sol} \rightleftharpoons \text{gel}$).

Pantin thinks that the temperature then affects the rate of this change of state as it does the rate of most other biological processes. This does not preclude the possibility that the source of the energy of

ameboid movement is ultimately a chemical reaction and in this respect Pantin and Rogers are in a direct agreement.

MATERIAL AND METHOD

The amebas used in this investigation when it was first started at the biological laboratory at Cold Spring Harbor*, were cultured from a strain found in the trough as it will hereafter be called. The trough is a wooden structure built near the laboratory for the purpose of raising amebas. The trough was supplied with running sea water. Other cultures were taken from strains found in the inner harbor near the laboratory.

When this experiment was continued at the University of Kansas the following September the amebas were successfully raised in large quantity from the sea-weed, Fucus, and sea water from the east coast.

The individuals studied were Mayorella conipes, Schaeffer '26, a small marine ameba, measuring on the average about 45 μ . Actively moving amebas were always chosen for each experiment and an attempt was made without actual measuring to use amebas of a uniform size.

After many observations on Mayorella conipes it was found that the active forms, under optimum conditions, move forward in the shape of a cone or wedge. The amebas move in a wavy path with the broad serrated end of the wedge anterior. Mayorella conipes does not form pseudopods as do other more common amebas but progresses in a mono-podal condition. One of the chief reasons for choosing this ameba lies in this fact.

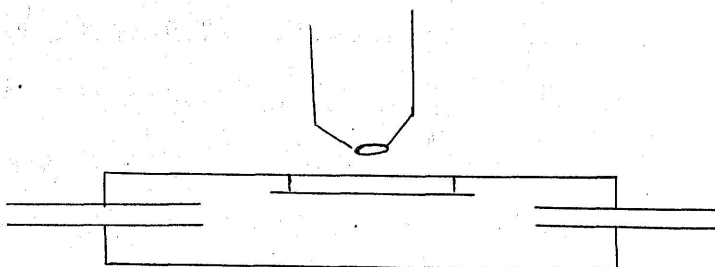
- - - - -

* This problem was started in the summer of 1926 at the Laboratory at Cold Springs Harbor, Long Island, N. Y. under the direction of Dr. A. A. Schaeffer.

METHOD

1. CONTROL OF TEMPERATURE.

In order to measure the rate of locomotion of Mayorella conipes under known conditions of temperature the Pfeiffer warming stage was first tried. (Fig.1.)

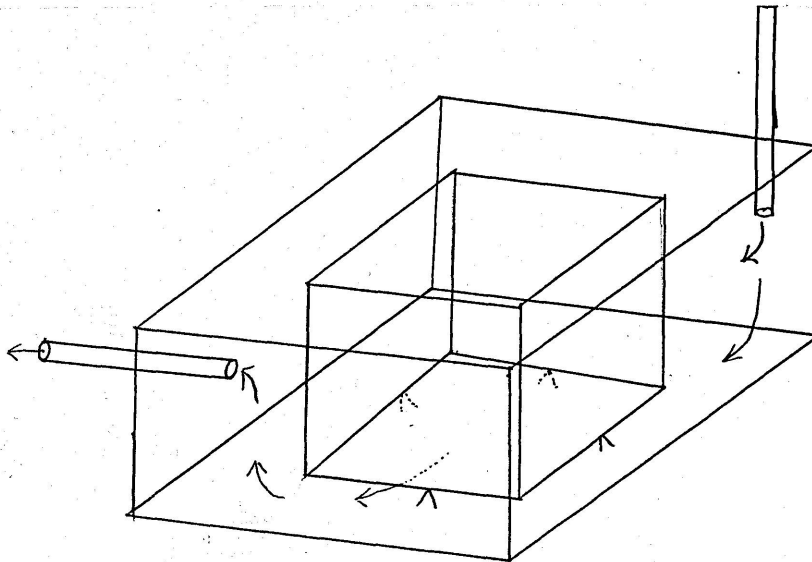


This consists of a shallow depression cell that holds the amoeba and cultural fluid with water at the desired temperature circulating beneath it. This method was soon discarded because (A) the depression cell was shallow and it was impossible to observe an amoeba for long periods of time without evaporation of the cultural fluid which would in turn concentrate the sea water and kill the amoeba; (B) one never knew exactly the temperature in the depression cell but had to assume it was the same as the circulating water, which may or may not have been correct; (C) in making long paths of the amoeba, the depression cell was found to be too small. The amoeba would reach the edge before the experiment was completed.

Schwittalla ('20) however, used the Pfeiffer stage with success by measuring the temperature with an electro-thermometer described by Hill ('12)

and Rodgers and Lewis ('14). This method is quite complicated and since the required apparatus was not at hand another method was used.

The apparatus consisted of two chambers, made of glass and held together with Dekhotinski cement. One chamber was designed to fit inside the other and elevated 12 mm. The outer chamber was equipped on the left side with an outlet pipe made of glass. The intake pipe was on the opposite side (right) leading from a three gallon jar. The inflow of water was controlled by a screw clamp. By releasing the screw clamp the water would flow around and under the deep inner chamber, which contained the amebas and the cultural fluid (fig.2.)



2. MEASURE OF TEMPERATURE

The temperature of the culture medium in the inner chamber was read directly from a centigrade thermometer, the bulb of which was submerged in the medium. By controlling the inflow with the screw clamp the desired temperature could be maintained for long periods of time (two to five hours) with a variation of not more than 0.5°C .

3. MEASURE OF TIME..

At first an Illinois 17 jewell watch was used. In the course of the experiment the Illinois watch was broken and replaced by a Hampton 15 jewell watch. In both cases the second hand was found to be well synchronized with the minute hand. The lag was only a few seconds in the course of any one experiment.

4. MEASURE OF DISTANCE.

In observing *Mayorella conipes*, a compound microscope with a 16 mm. objective and a 10x ocular was used. With the aid of a camera lucida drawings were made at various intervals depending on the temperature of the medium. At low temperatures the ameba moved slowly and drawings were made two, three and sometimes four minutes apart. At the higher temperatures when the ameba moved faster, the drawings were made each minute and sometimes every half minute. During the course of any one experiment, however, an attempt was made to keep the time interval constant for any one temperature.

5. METHOD OF COLLECTING DATA AND MAKING GRAPHS.

In each experiment an ameba was placed in the inner chamber in its own dialized cultural medium. Water from the three gallon jar was then circulated around and under this inner chamber. The temperature was kept constant or made to fluctuate according to the nature of the experiment. In either case camera lucida drawings of the ameba were made at definite intervals.

In the experiments where the temperature fluctuated, the usual starting point was 15°C or 20°C. This temperature was kept constant for 30 minutes and a track of the ameba was made. The temperature was then raised 5°C, and a period of 15 minutes was allowed to elapse so the ameba

could become acclimated to the new temperature.

This process was repeated, each time raising the temperature 5°C above the preceding temperature and each time allowing for acclimatization until all movement in the ameba stopped.

The tracks of the amebas at the various temperatures were now measured exactly to the nearest half millimeter. By dividing the total length of the track by the total time at any one temperature the rate in millimeters per minute was obtained for that temperature.

Graphs were now made for each ameba, plotting the rate against the temperature in each case. By a study of these graphs it will be found that the velocity of this ameba varies definitely with the temperature. These graphs bring out some other very interesting processes in connection with ameboid movement that will be taken up in detail in the course of the experiment.

The distances that the ameba moved as expressed on the graphs in millimeters is the apparent distance as it appeared under my optical combination. In order to obtain the actual distance the ameba moved in any case it is necessary only, to multiply by 5.45. (1 mm. on graph = 5.45)

RESULTS

It has been demonstrated by Schaeffer ('25) working with *Cochliopodium* and Pantin ('24) working with two limax forms that the velocity varies with the temperature in a manner similar to many other biological processes. Pantin points out that the velocity is greatest at an optimum temperature above which something ^{thing} ~~times~~ it is destroyed. That is, when the temperature is increased, the velocity is increased up to a certain point. Beyond this point (optimum) an increase in temperature tends to destroy something, probably a protein, in the ameba and checks its velocity.

Schaeffer's work with *Cochliopodium* followed very close to Pantin's.

The problem of ameboid movement is quite extensive and it is necessary that we have a large amount of data on the various groups of amebas as a basis for a complete investigation of this subject.

Since the limax forms used by Pantin (24) and the *Cochliopodium* used by Schaeffer belong to such widely separated families, but behave so nearly alike, it was necessary, as well as interesting, to know what another family of amebas would do under similar conditions.

The family of amebas chosen for this investigation was Mayorellidae, a group that stands about midway between the limax type (*Chaidae*) and the *Cochliopodium* (*Haylodiscidae*).

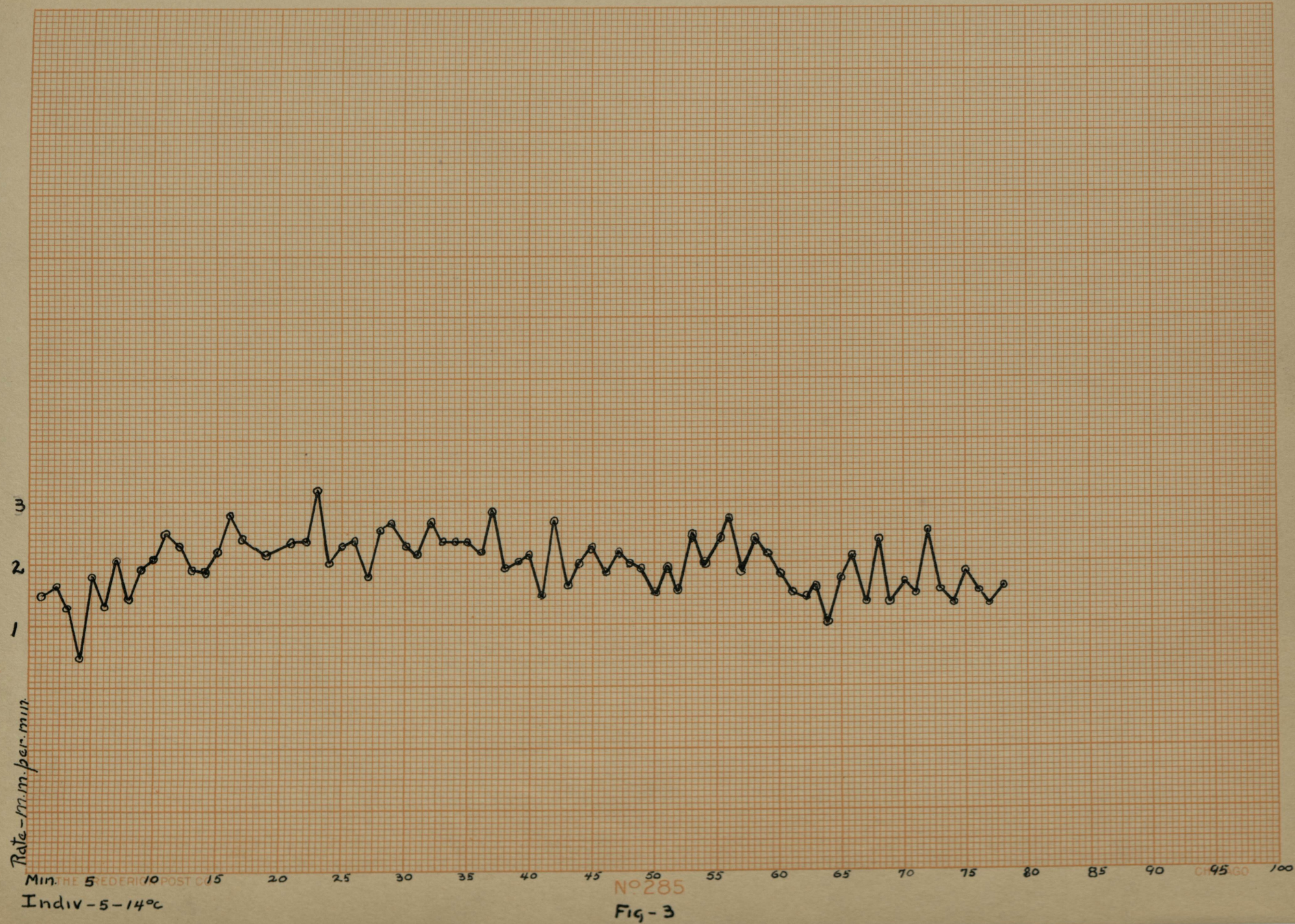
REACTION OF MAYORELLA CONIPES AT CONSTANT TEMPERATURES.

1. AT LOW TEMPERATURES.

The rate at which *Mayorella conipes* moves when it is kept at a constant ^{Temperature} ~~rate~~ varies considerably depending on the temperature. At low temperatures (14°C) the up and down range of the temperature-speed curve was found to be from 0.5 mm. per minute to 3.15 mm. per minute at the extremes. The greatest fluctuation within one minute being at the rate of from 0.5 mm. per minute to 1.75 mm. per minute. Most of the fluctuations, however, were much less. Fig. 3 shows the complete graph for individual No. 5 observed over a period of 77 minutes at 14°C.

2. AT HIGH TEMPERATURES.

At high temperatures (35°C) the up and down range in the curve was found to be much greater. Frequently, in one minute the rate increased from 4.5 mm. per minute to 12 mm. per minute in one minute, and in one



instance the rate increased from 2.5 mm. per minute to 15 mm. per minute in one minute.

Fig. 4 shows the curve for individual No. 10 at 30°C, 35°C, and 37°C, respectively. By comparing this graph with the one shown in Fig. 3 the difference in the fluctuations at the high and low temperatures can be seen at once.

This small fluctuation at the low temperatures as compared with the great amount of fluctuation at the high temperatures is an interesting feature of ameboid movement, chiefly, because of all the work that has been done on ameboid movement it has never before been described. It is something entirely new in connection with ameboid movement and may lead to some very interesting physiological experiments. It undoubtedly has a direct bearing on the mechanics of ameboid movement, and as yet no theory so far brought out is capable of explaining it. This is just another case showing the futility of establishing a theory of ameboid movement before we know the important facts concerned. Any theory attempting to explain ameboid movement must take into account (1) that an ameba does not move at a uniform rate at a constant temperature and (2) that at high temperatures the rate of movement is less uniform than at low temperatures.

Fig. 5 is offered as proof of this statement, being based on the temperature-speed curves of eleven amebas, picked more or less at random from the data collected. These graphs are exactly like those in Fig. 3 and Fig. 4 except that they have been condensed by taking an average of each five points on the original graph and plotting that average as one point.

The three lower graphs show the more nearly uniform rate at low temperatures as compared with the very irregular rate at high temperatures

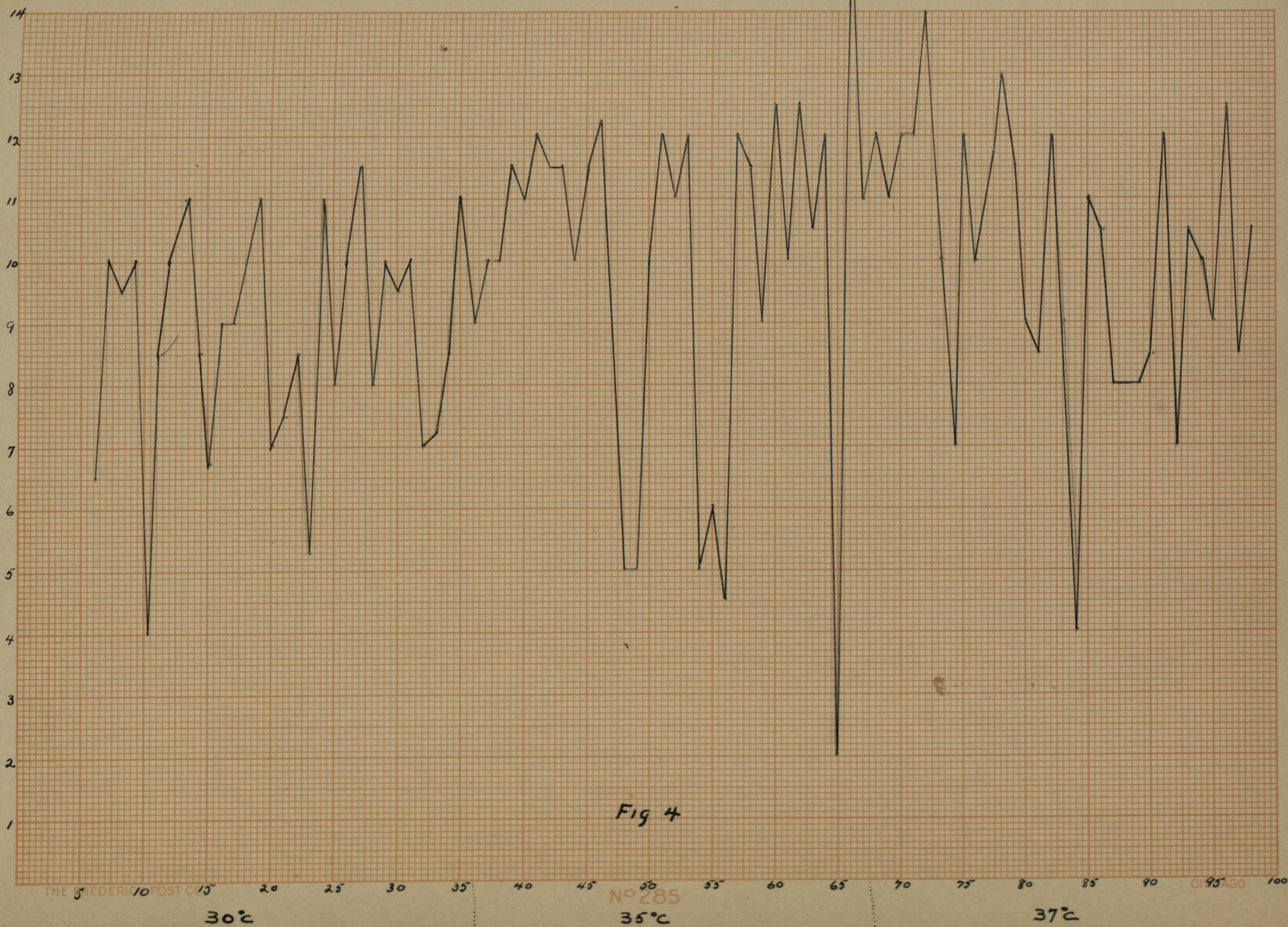


Fig 4

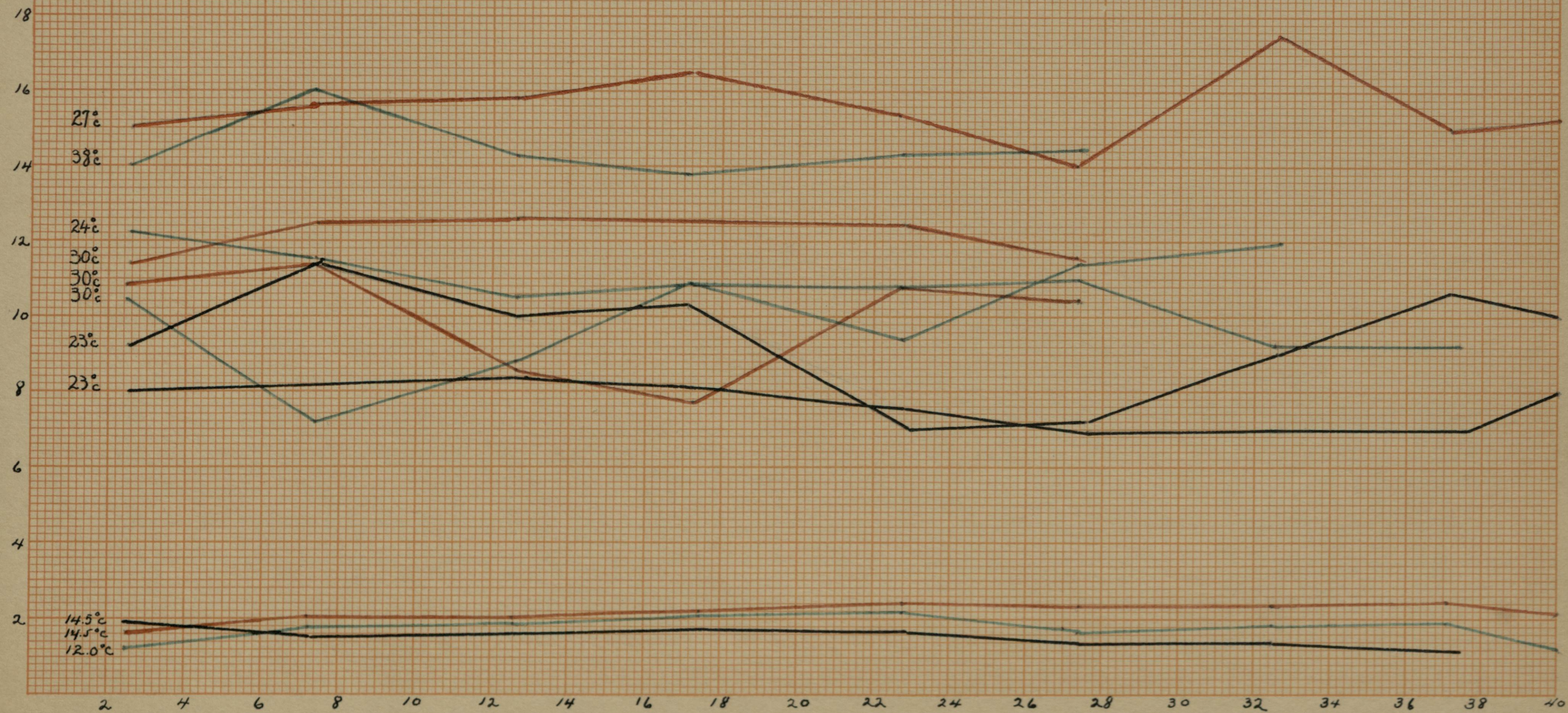


Fig 5

near the optimum.

The rate of movement of Mayorella conipes, at constant temperatures, differs considerably from that of the limax forms used by Pantin. He found that when they (limax) were kept at a constant temperature, they maintained a constant velocity within 1% to 5%. This, if plotted would make almost a straight curve. In the limax experiments a ghost micrometer was used to measure the velocity by timing the ameba as it moved over a given number of divisions, with a stop watch. This method was not used in working with conipes because it was found that more accurate measurements could be obtained from a continuous camera lucida path over a long period of time. Had Pantin's method been used the fluctuations in the rate of movement in the ameba could not have been detected.

In measuring the velocity of an ameba with a ghost micrometer over a short period of time it is entirely possible to get all "ups" or all "downs" or mostly one or the other. In short, observations and camera lucida drawings every minute or every two minutes, over a period of an hour or more undoubtedly gives a more accurate account of what the ameba is doing, at every minute of the experiment, than would observations at ten minute intervals, with no record of what goes on in between.

VELOCITY AT VARIOUS TEMPERATURES OBSERVED ON THE SAME AMEBA.

After having determined the reaction of the ameba at constant temperatures it became of interest to know what the same ameba would do at different temperatures. This necessitated a slightly different method. An ameba was selected and placed in the inner chamber. The usual starting point was 15°C, or 20°C. A track of the ameba was made with the aid

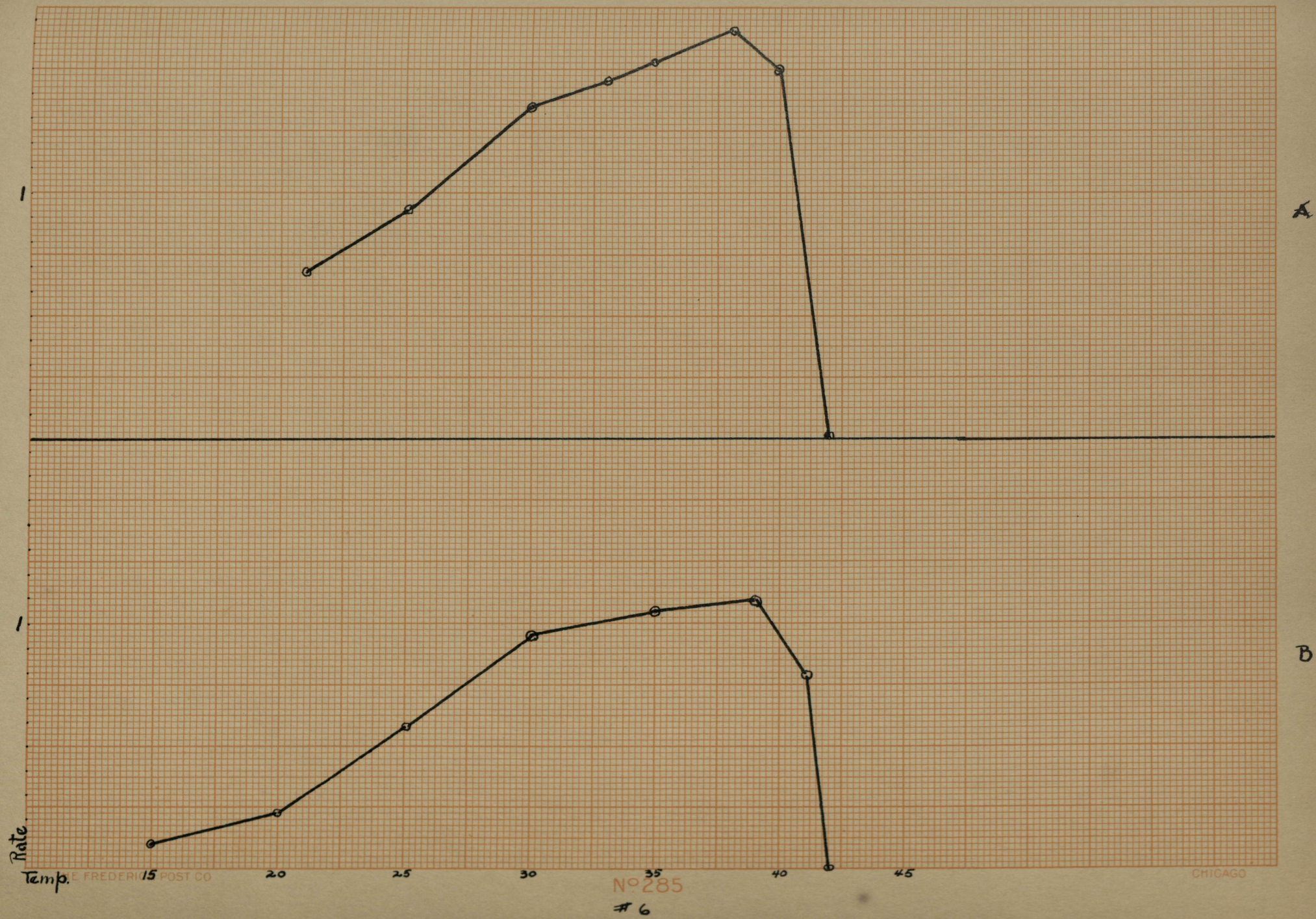
of a camera lucida, for a period of 30 minutes. At the end of this time the temperature was raised 5°C and a period of 15 minutes was allowed to elapse so the amoeba could be acclimated to the new temperature. This process was repeated, each time raising the temperature 5°C above the preceeding one and allowing each time for acclimitization, until such a high temperature was reached that all movement in the amoeba stopped.

A graph was now made according to the method previously described. This graph can be seen in Fig.6. This graph shows the rate of movement plotted every two minutes, for 247 minutes for individual No.9. Fig.7 shows the original of one of the graphs before it was reduced.

Sub- Optimal Temperatures (15°C --- 38°C , 15°C --- 39°C)

At temperatures below the optimum the amoeba moves in a regular, wavy, sinoidal path, with a more or less uniform increase in rate, as the graph will show, (fig.6) until a temperature of about 30°C is reached. Above this temperature the rate of increase of velocity falls rapidly until the velocity reaches its optimum at 38°C . is reached. Above this temperature the rate of increase of velocity falls rapidly until the velocity reaches its optimum at 38°C . It is interesting to compare this optimum with the extremely low optimum of the limax forms, which Pantin found to be 20°C for type B, and 22°C and 25°C for type A. After the optimum had been reached in type B, at 20°C , the velocity fell rapidly to zero at 26°C , but death did not occur until 30°C had been reached. It is extremely interesting to note that Mayorella conipes was found to have an optimum temperature actually above the death point of the limax forms.

The low optimum of the limax forms does not appear to be general for all cases of ameoid activity. Muscle reaches its optimum activity



between 28°C and 30°C and at temperatures above the optimum the activity of the muscle decreases rapidly. McCutcheon has shown that the optimum for human leucocytes occurs at 40°C as might be expected.

There is a possibility that the temperature of the environment might determine the optimum of the amebas as suggested by the human leucocytes. But when we consider that the amebas used in this experiment have been cultured in the laboratory at a room temperature that will average about 23°C, and that they still have the same optimum temperature now as they had when taken directly from the natural habitat, it seems that the temperature of the environment of an organism is not sufficient to account for its optimum.

In comparing the graphs in Fig.6 it will be seen that the shapes of the graphs in both cases are very similar, and that in both, the velocity increases rapidly up to 30°C, and beyond that temperature, the rate of increase in velocity falls rapidly until the optimum is reached. There is a slight difference in the optimum of the two amebas shown by the graphs in Fig.6. In A the optimum is 38°C, while in B it is 39°C. The fall in the velocity above the optimum in each case is similar. In spite of the fact that these amebas are very much alike in their reactions to the various temperatures, they are consistently different in their rate of movement. Individual 9 is consistently faster than individual 10. At 25°C, individual 9 is going 2.84 mm. per minute faster than individual 10, and at 30°C individual 9 is going 3.40 mm. per minute faster than individual 10, while at 35°C individual 9 is going 3.76 mm. per minute faster than individual 10. This, with additional data, can be seen in the following table. This is not an isolated case but occurred many times throughout the

experiment.

Graph	25°C	30°C	35°C
A	8.22	12.15	13.74
B	5.38	8.75	9.68
Diff.	2.84	3.40	3.76

FALL IN RATE ABOVE THE OPTIMUM.

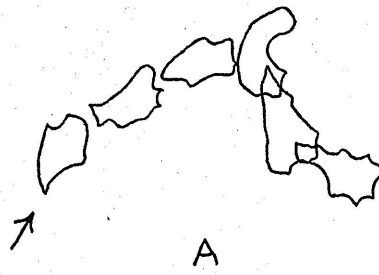
After the ameba has reached its optimum, the velocity falls rather rapidly to 41°C and from this point the velocity falls almost immediately to zero at 42°C. At temperatures near the optimum, and sometimes above, the movement of the ameba differs slightly from that below the optimum, in that at more or less regular intervals, the ameba assumes a very characteristic crescent shape and moves off in every instance at nearly a right angle to its former path (Fig.8).

This seems to be a racial character rather than an individual one. Pantin noticed in his limax forms a similar irregularity in the movement above the optimum, but it was characterized by the formation of lateral pseudopods rather than crescents. It is interesting to note too, that below the optimum the limax forms studied take a straight path while Mayorella conipes takes a wavy one characteristic of most other amebas.

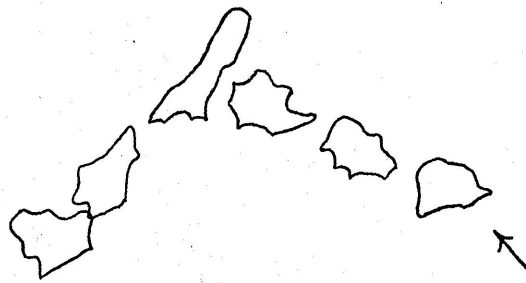
THE EFFECT OF SUCCESSIVE RISES AND FALLS IN TEMPERATURE

After observing Mayorella conipes at constant temperatures and at gradually increasing temperatures it became of interest to know if the velocity at any given temperature below the optimum was fixed or depended upon the previous temperature.

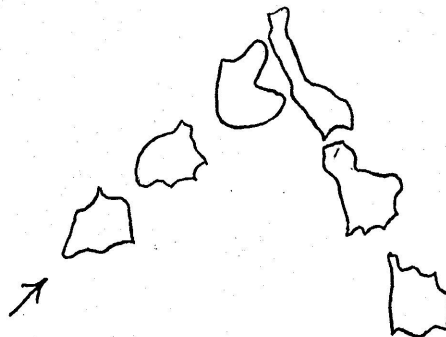
In order to determine this, an ameba was selected and observed for 15 minutes at 25°C. The temperature was now lowered to 20°C and 10 minutes allowed to elapse so the ameba could be acclimated to the new tem-



A



B



C

Fig 8

perature. The ameba was now observed for 15 minutes more and the temperature this time was raised to 15°C ; acclimated for 10 minutes and observed. This process was continued up to 25°C ; allowing first 10 minutes at each temperature for acclimatization and observing for 15 minutes. This has been tried on other amebas, but never on *Mayorella conipes*, and never on any for as long as five hours and twenty-five minutes at a time.

The velocity at each temperature was determined. Fig 9 shows four of the longest observations plotted together on a single graph. For the limax forms, Pantin found a distinct "lag" in the velocity obtained by a rising temperature. That is, he found the velocity at 15°C for example to be higher if approached from high temperature, than if approached from a low one.

Graph D, Fig. 9, shows a distinct lag. When approached from a low temperature the ameba was going 2.6 mm. per minute at 15°C , but when approached from a high temperature it was going at the rate of 618 mm. per minute at 15°C . Again at 20°C when approached from a low temperature it was going 5.4 mm. per minute, but when approached from a high temperature it was going 7.4 mm. per minute at 20°C . This curve shows only one "up" and one "down", and this is all that has been recorded before by any observer.

In graph A. Fig. 9, the "lag" is very definite if we consider only the first "down" and "up". Likewise, in graph C, if we observe only the first "up" and "down" the lag is very distinct, but if we observe succeeding "ups" and "downs" over a longer period of time the idea that the velocity is greater when approached from a high temperature than when approached from a low one does not hold.

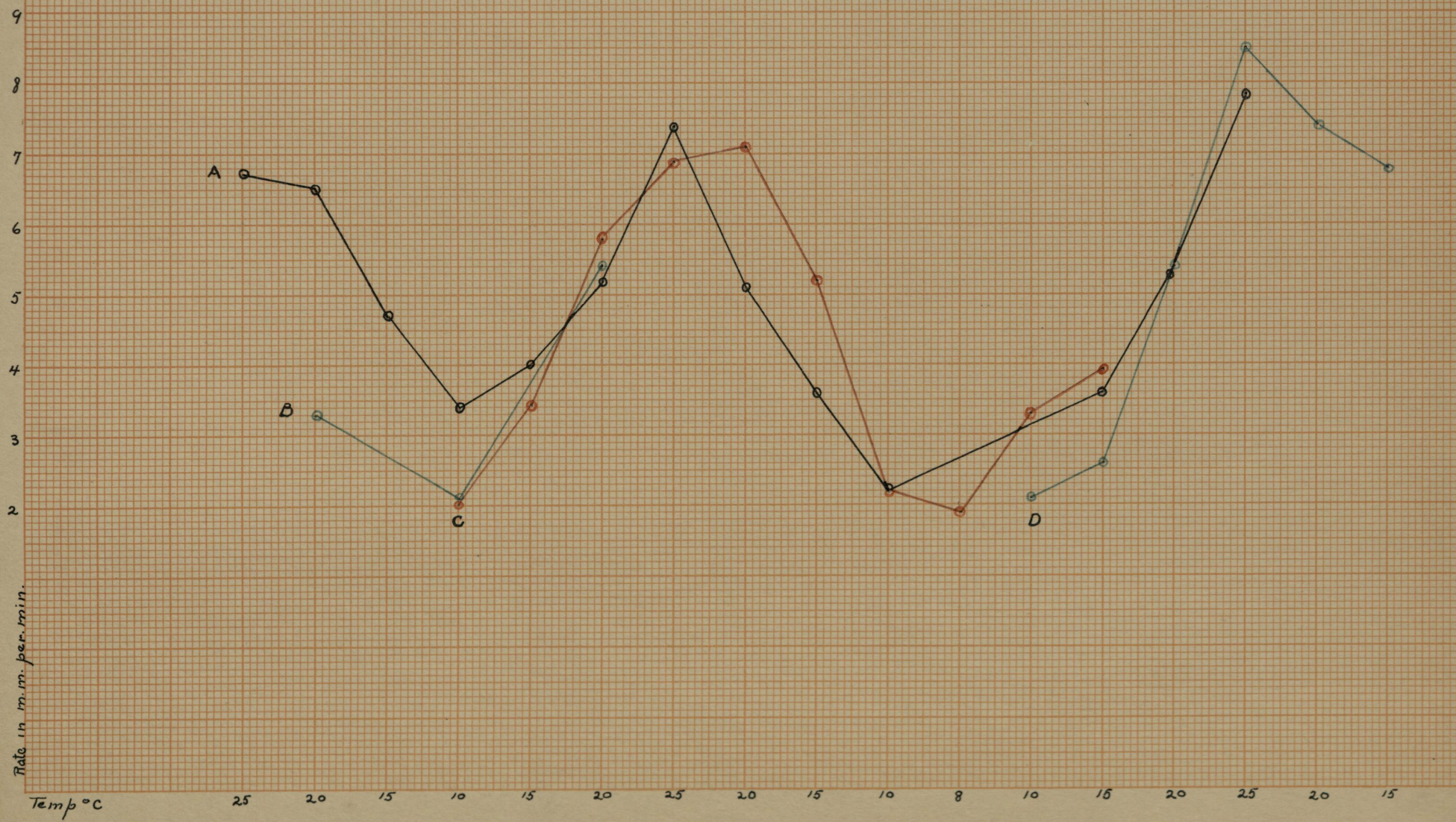


Fig 9.

Pantin's idea of lag is not as general in application as might be supposed since altho it may occur in the first part of a curve of *Mayorella conipes*, it does not occur in succeeding curves.

In these experiments the optimum was never reached, 25°C being the the highest temperature and accordingly nothing should have been destroyed in the ameba, according to Pantin. The only possible explanation for lag is that the acclimatization period was not long enough between successive changes in temperature. But on the other hand there is evidence to show that in other marine amebas the velocity usually becomes constant in about one minute after a change in temperature.

These experiments show that the previous history of the ameba must be taken into account and that the rate of movement of the ameba at any given temperature cannot be taken solely as an expression of its physical state at that temperature, regardless of its previous temperature history.

RHYTHMIC ACTIVITY.

In the study of this ameba it became necessary to investigate that phase of ameboid movement that has to do with rhythmic activity. Schwitalla ('24) speaks of the "rhythmic character of the changes in the locomotor rates" in the amebas studied by him. This led to an investigation of this point to see if the rhythmic character that Schwitalla spoke of was present in Mayorella conipes.

The word rhythm comes from the Greek (*ρυθμός*), meaning "measured motion". We find the word in connection with music, poetry, prose, fine arts, physics, pathology, and various biological processes. Calkins ('04) and Mast ('10), Schwitalla ('24) and Woodruff have called attention to the rhythmic activity of amebas and other protozoa.

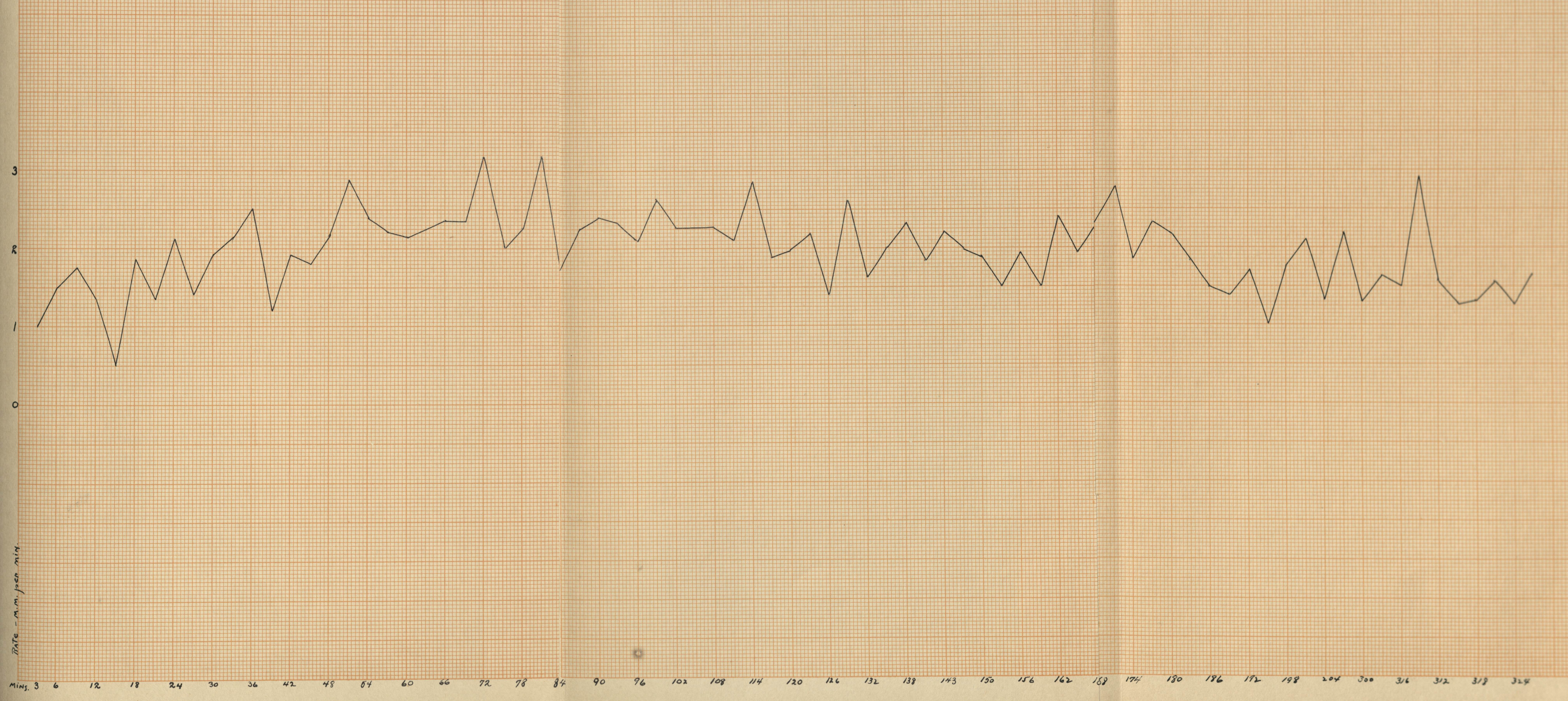
While the definitions for rhythm are numerous, there is an idea running throughout them that makes them essentially the same, that being, that for any process to be rhythmical in its action, that action must recur at regular periods and must be predictable as in the case of such biological processes as heart beat and muscle contraction. In each of these cases an action occurs, then recurs regularly, such that it can be predicted fairly accurately. This, according to a composite of all the definitions, can be called rhythm. With this idea of rhythm in mind we will examine the experimental data.

Two species of amebas were used in this phase of the experiment. Mayorella conipes was the one used in the previous work and the first one used here. Because Mayorella conipes has no pseudopods*, a second species having pseudopods was used as a basis of comparison (Chaos diff-luens Muller).

The amebas were kept and observed at constant temperatures and camera lucida drawings and graphs were made according to the method previously described.

By observing Fig.10 of Mayorella conipes, it will be found that the change of the locomotor rate is not rhythmical according to the definition of rhythm, because throughout the entire curve of 324 minutes in length, there cannot be found, even for a small portion of the curve, an action that recurs predictably. By comparing this graph of the ameba with a kymograph record of the normal heart beat of a frog, it will be seen at once, by the character of the curve, that there is a difference between a rhythmical (frog heart) and a non-rhythmical (ameba) action. Over 30 paths were made of this ameba at constant temperatures and all are similar to the one shown in Fig.10, 324 minutes long. Considering rhythm as it is

* Mayorella conipes has very small pseudopods but they do not determine the direction of movement as to the pseudopods in larger amebas.



generally spoken of in connection with other biological processes, (heart action, muscle contraction) and as it has been defined, it can be safely said that the locomotor rate of Mayorella conipes is not rhythmical.

In general it can be said that Mayorella conipes moves with alternated accelerating and retarding phases. These phases are seldom of the same duration and at any minute it is impossible to predict the action of the ameba for the next minute.

It was noticed, however, that occasionally Mayorella conipes would elongate and assume a crescent shape. This crescent consisted of two pseudopods with protoplasm streaming forward in each. This caused the ameba to go slower for a time until one pseudopod was retracted and the other predominated. After this, the speed of the ameba increased. In every instance there was a marked correlation between these crescent forms and the decrease in rate of locomotion. From these observations it became quite evident that the decrease in rate was due to the formation of pseudopods. Before one could be certain that this was the case it was necessary to make the same observations on an ameba of a different species having a large number of pseudopods. If then, the decrease in rate was due to the formation of pseudopods, and was common to all amebas it should show up very markedly in this ameba. The ameba chosen for this investigation was Chaos diffluens one of the common large amebas, known to form numerous pseudopods. In order to compare it directly with Mayorella conipes it was treated in a similar manner, viz;- the same constant temperature and camera lucida drawings made every minute.

It was found upon examining the path of this ameba, a portion of which is shown in Fig.11, that there was a considerable variation in the rate, and too, just as in Mayorella conipes there was a marked positive

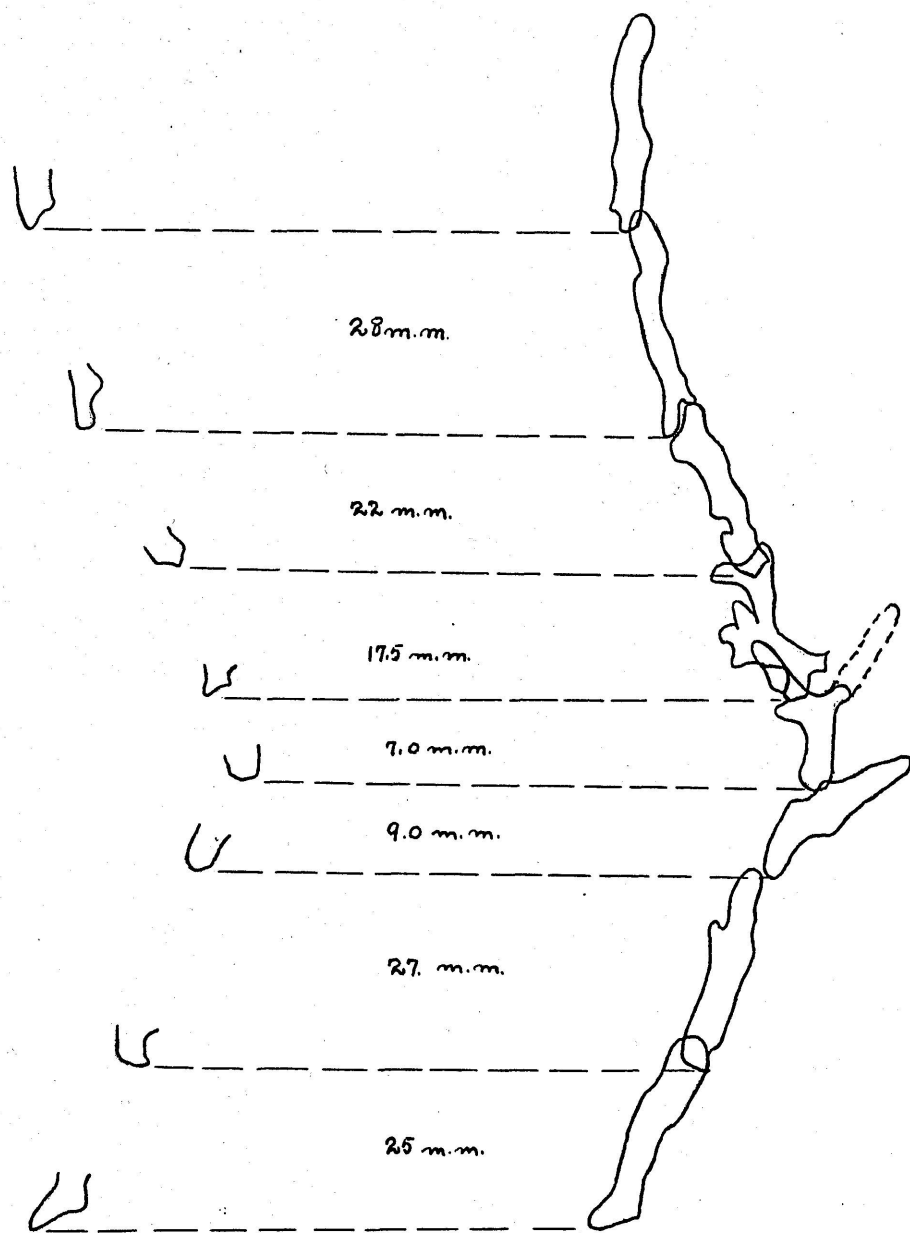
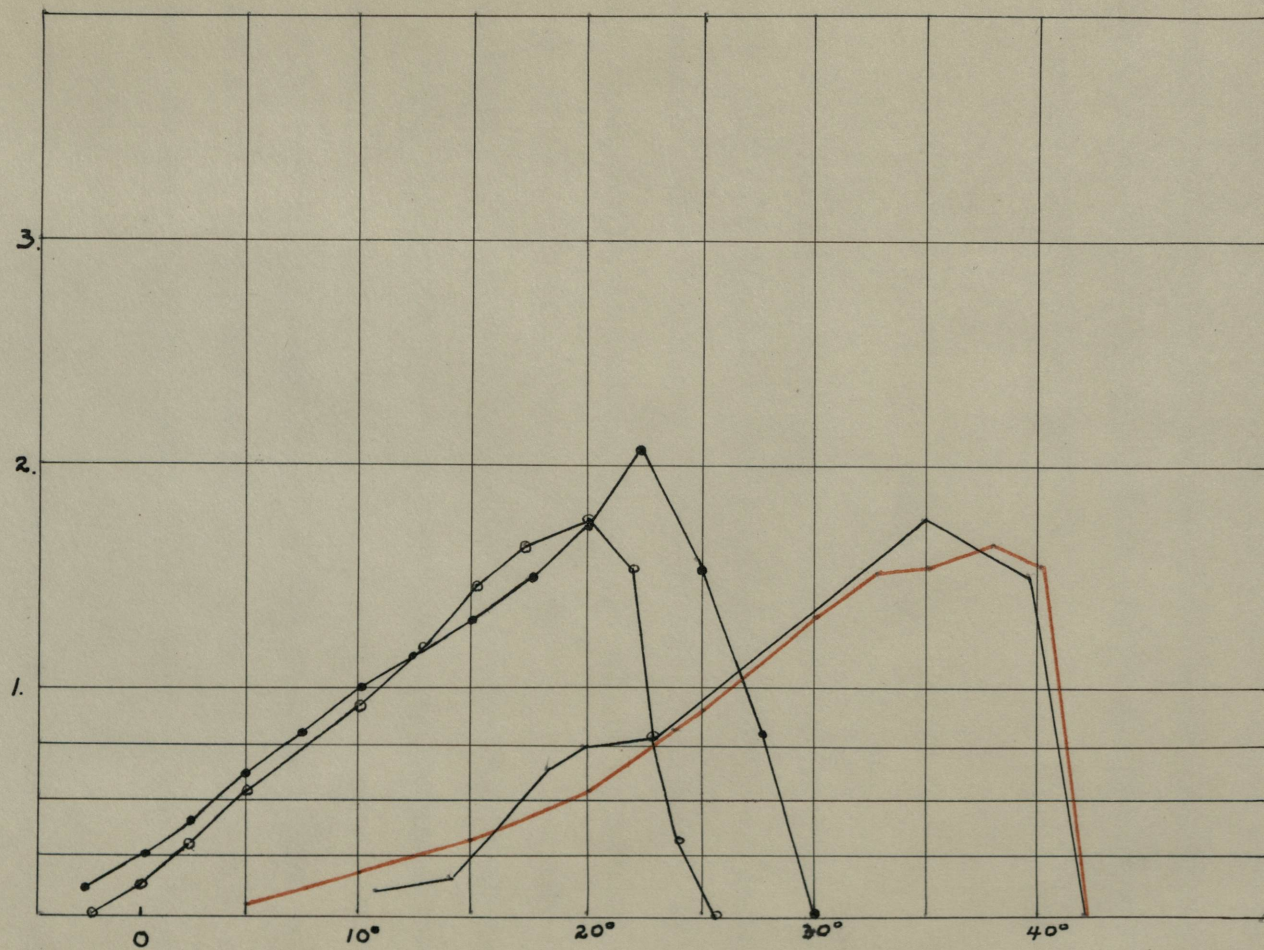


Fig 11



correlation between the decrease in rate and the formation of pseudopods.

Schwitalla (24) in his study of the movement of amebas drew only the posterior ends as Fig. 11 will show, and he did not observe the pseudopods. Since there is a marked positive correlation between retardation and pseudopod formation in the two species of amebas studied here, it is highly probable that the retardation phases observed by Schwitalla were due largely to pseudopod formation.

CONCLUSIONS

1. When Mayorella conipes is kept at a constant low temperature (14°C) for long periods of time (60 to 214 minutes) the change in rate of movement is much less than at high temperatures (35°C). The range at the low temperatures was from 0.5 mm. per minute to 3.25 mm. per minute while in the high temperatures it was from 4.5 mm. per minute to 12.5 mm. per minute. The greatest uniformity in movement was found to be at the low temperatures (14°C); as the temperature was increased the rate of movement became less uniform as the optimum was approached.

2. The optimum temperature for this ameba was found to be between 38°C and 39°C . The optimum for this ameba was found to be actually above the death point of Pantin's limax forms. (Type A, 20°C . Type B, $22^{\circ}\text{--}25^{\circ}\text{C}$).

3. With an increase in temperature, the velocity increased until the optimum was reached (39°C). Beyond this point something was destroyed, probably a protein, (Pantin) and the ameba failed to respond by an accelerated rate of movement to a further increase in temperature. The ameba recovers if the temperature is kept below 42°C . In this respect, conipes,

corresponds to other amebas that have been observed.

4. At temperatures near the optimum the ameba assumes a characteristic crescent shape. This is a response on the part of the ameba to temperatures close to the optimum, and has been interpreted as an attempt to form pseudopods. In larger amebas, where large pseudopods normally occur, a similar response is always made to temperatures near the optimum and is always accompanied by the formation of many pseudopods. This seems to be a racial character rather than an individual one. It occurred in every instance and has been observed in other members of the family Mayorellidae.

5. The idea that the velocity is greater when approached from a high temperature than when approached from a low one does not hold for Mayorella conipes. Occasionally there may be a distinct "lag" under the above conditions but that it is not a permanent response on the part of the ameba is shown by the fact that there are just as many cases where the "lag" does not occur where it rightfully should be expected.

6. These experiments show that the previous history of the ameba must be taken into account and that the rate of movement of the ameba at a given temperature cannot be taken solely as an expression of its physical state at that temperature, regardless of its previous temperature history.

7. According to the common definitions of rhythm and as we know it in connection with other biological processes, it can be said that the rate of movement of Mayorella conipes and Chaos diffluens are not rhythmic. Both of these amebas have periods of acceleration and retardation but these were not found to occur rhythmically and when they did occur were due to the formation of pseudopods in the retarding phases and lack

of pseudopods in the accelerating phases. This point was not detected and in fact could not have been detected by Schwitalla since he drew only the posterior ends of the ameba. The reasons for the retarding and accelerating phases are to be found only by knowing the condition of the pseudopods. It seems probable that these phases can be understood only by studying the entire outlines of the amebas and that nothing can be learned by drawing only the posterior ends.

Literature List.

Bullington, W.W. 1925. A study of spiral movement in the ciliates infusoria. Arch. F. Protistenk., Vol 50, pp 219-274.

Dellinger, Jo. Exp Zoo Pg. 366

Mast, S.O. 1923. Mechanics of locomotion in Ameba. Proc. Nat. Acad. Sci., Vol. 9, pp. 259-261.

Mast, S.O., and L.C. Pusch. 1924. Modification of response in ameba. Biol. Bull., Vol. 46, pp. 55-59

Mast, S.O. 1926 Structure, Movement, locomotion and stimulation in amoeba. Jour Morph and Physiol. Vol. 41. pp. 327-425.

McCutcheon, M. 1923 Studies on the locomotion of leucocytes 11, Am. Journ. Physiol. Vol. 66, pp.185.

Pantin, C.F. A. 1924 On the Physiology of Amoeboid Movement 1. Jour. Marine Biol.

Pantin, C.F.A. 1924. On the physiology of ameboid movement. 11. Brit. Jour. Exp. Biol., Vol. 1, pp. 519-538.

Schaeffer, A.A. 1920. Ameboid movement. vii 156 pages. Princeton Press.

Schwitalla, A.M. 1924. The influence of temperature in the rate of locomotion in amoeba. Jour. Morph. Vol. 39, pp. 465.

Schaeffer, A.A. 1926 The taxonomy of the amebas with descriptions of thirty-nine new marine and fresh-water species. Publ. Carnegie Institution of Wash. No 345 (Contains full bibliography of previous papers by this author).

Schaeffer, A.A. 1926. Recent discoveries in the biology ameba.

Snyder, 1906	Am. Jo. Phys 17 No. 4
1908	Am. Jo. Phys. 22 No. 1
1908	Am. Jo. Phys 22 No. 309
1911	Am. Jo. Phys 28 No. 167

Snyder	Univ. Calif. Physiol 1905 No. 15 125
--------	--------------------------------------